that human PARG domains will closely resemble and therefore also have a fold based on the macrodomain. The key sequence motifs mentioned above are highly conserved and a homology search for human PARG (http://www.sbg.bio.ic.ac.uk/~phyre/) indeed confirms the close relationship to *T. curvata* PARG.

Important questions remain open. Only a subset of the macrodomains are catalytically active and some are not even capable of binding ADP-ribose or related nucleotide ligands (Kustatscher et al., 2005). For example, the ligands for the histone variants macroH2A1.2 and macroH2A2 remain completely unknown, despite the high conversation of these histones across vertebrate evolution. On the issue of PAR degradation as a regulatory posttranslational modification, the question of which enzyme(s) may specifically remove the "final" ADP-ribose moiety from posttranslationally modified proteins remains open. The hunt for such enzymes and for physiological PARP-family targets is made more complex by the fact that there is evidence to support both glutamate and lysine residues as key ADP-ribose acceptors. There can be much confidence, however, that such open questions will soon be addressed.

In conclusion, after more than forty years of research into ADP-ribosylation signaling, the paper by Slade et al. (2011) has provided us with detailed structural insight into PARG enzymes, a plausible PAR degradation mechanism, and revealed a surprising relation to the macrodomain module. As the ADP-ribosylation field shifts into a higher gear with this and other recent progress, the stage looks set for further surprises. Other macrodomains in disguise may abound, promising to reveal new molecular and physiological roles for this nucleic acid with signaling functions.

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It Takes Two to Get3

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Tail-anchored (TA) membrane proteins perform essential cellular functions. They are posttranslationally inserted into the endoplasmic reticulum (ER) membrane by interaction of the Get3 chaperone with the Get1/2 receptor. Two independent structural and functional analyses of the Get3/receptor complex by Stefer et al. and Mariappan et al. now provide insights into TA protein insertion.

In the textbooks, insertion of membrane proteins into the ER is mediated by the universally conserved signal-recognition particle (SRP), which relies on the presence of an N-terminal signal sequence (Grudnik et al., 2009). In eukaryotes, however, about 5% of all membrane proteins, including the SNARE or Bcl-2 family proteins, carry their targeting signal within a single transmembrane domain present at their C terminus and are therefore termed tail-anchored (TA) proteins. They are subject to the recently identified GET (guided entry of TA proteins) pathway (reviewed in Simpson et al., 2010). The GET machinery comprises at least five components (Get1–5) that mediate the three main steps of TA protein insertion: Get4/5 assisted loading of the Get3 ATPase with a TA protein, docking of the Get3/TA protein complex to the Get1/2 receptor at the ER, and subsequent insertion. The Get3 ATPase forms the core of the GET machinery, and a series of Get3 crystal structures suggests that the Get3 dimer oscillates between an "open" and a "closed" state by a nucleotide-dependent rotation of the two subunits (Simpson et al., 2010). While the dimer is clamped together at the bottom by a zinc ion, the TA protein is expected to bind to a hydrophobic pocket on top of the ATPase domain in the TA protein binding domain (TABD),

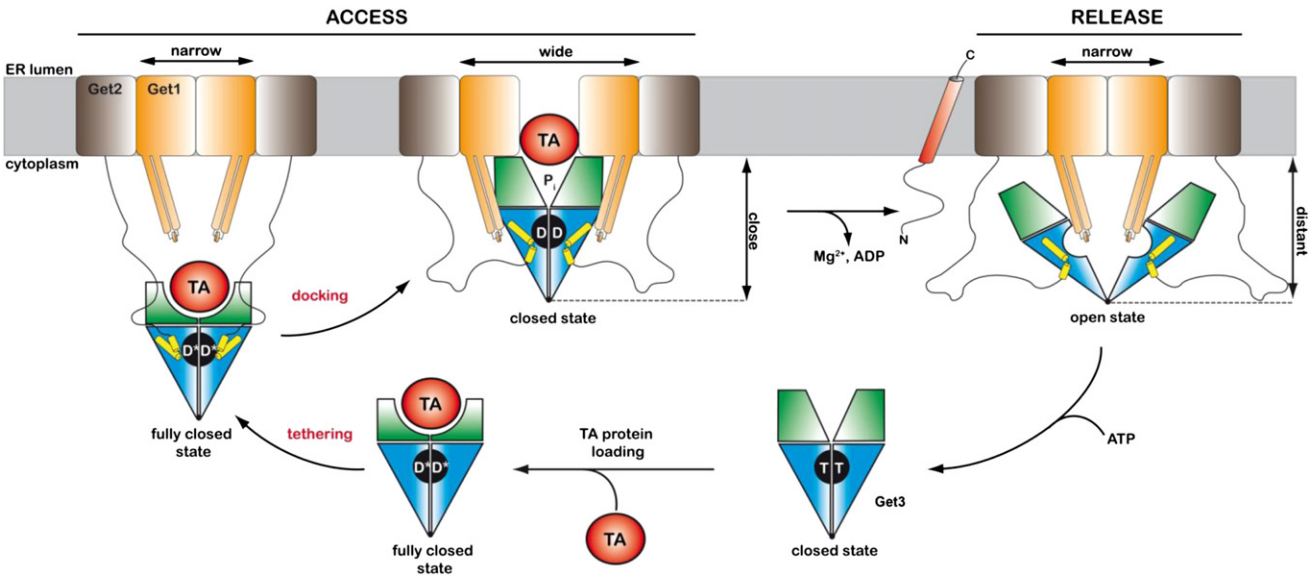

Figure 1. TA Protein Insertion by the Get System
Get3 consists of two domains, an ATPase domain (blue) and a TA protein binding domain (TABD, green). The Get3 dimer delivers TA proteins (red) to the membrane-embedded Get1/2 receptor (brown, orange). Targeting and insertion involves a series of conformational changes in the Get3/receptor complex (see text).

which is shaped in the presence of the transition-state analog ADP·AlF4$^-$ (Mateja et al., 2009). Membrane insertion of TA proteins then relies on the interaction of Get3 with the Get1/2 receptor complex at the ER membrane (Auld et al., 2006; Schuldiner et al., 2008; Wang et al., 2011). Although TA protein binding seems to induce ATP hydrolysis, the precise timing of ATP hydrolysis and how it is coupled to TA protein binding and release at the membrane is not known. Now, two independent studies (Mariappan et al., 2011; Stefer et al., 2011) shed light on the molecular framework of the decisive targeting and insertion of TA proteins. Together, we provide convincing evidence that the Get3-receptor interaction follows a two step mechanism with Get2 first tethering the Get3/TA protein complex to the membrane and docking to Get1, then allowing for TA protein release. Structural and functional data show that efficient targeting and insertion depends on nucleotides that induce the closed state of Get3 but allow for the transition to the open state, which correlates with ATP hydrolysis and, finally, nucleotide release at the membrane.

Briefly, the two studies show that Get1/2 together with the Get3-TA targeting complex provides a minimal and physiologically relevant system for TA protein insertion into the membrane and describe the structural basis of Get3 interaction with the Get1/2 receptor at the membrane. Get1 and Get2 are integral membrane proteins each comprising three transmembrane helices (TMDs) and a cytoplasmic domain (CD) required for TA protein insertion. A whole series of Get3 structures in complex with the CDs of either Get1 or Get2 are presented, visualizing different states of the Get3-receptor complex. The structures of Get3/Get1-CD show a symmetric heterotetramer with two Get1 molecules bound at the interface of a nucleotide-free, open Get3 homodimer. Get1-CD forms a coiled coil, which inserts like a wedge, interacts with both Get3 subunits, and might interfere with nucleotide binding. In contrast, the crystal structures of Get3/Get2-CD show that Get2 binds laterally to the dimer, contacts only one Get3 subunit in a nucleotide-bound, closed state, and does not interfere with TA or nucleotide binding. The presence of ADP-AlF4$^-$ (Mariappan et al., 2011) or ADP derived from ADP-aluminium fluoride (Stefer et al., 2011) in the two structures suggests that Get2 provides a first tether for the Get3/TA complex. Get1 and Get2 share adjacent and partially overlapping binding sites at Get3. This suggests that upon docking to Get1, Get2 is at least partly displaced from the initial tethering complex and that both could bind at the same time. Here, the two studies differ: while a fluorescence labeling study did not provide evidence for a trimeric complex (Mariappan et al., 2011), pull-down and NMR experiments clearly show that Get1 and Get2 can bind at the same time (Stefer et al., 2011). An additional structure of the Get3/Get1-CD complex representing a semiopen state of the Get3 dimer (Stefer et al., 2011) allows definition of two distinct interfaces between Get1 and Get3: independent of the conformational state of the Get3 dimer, the interface that overlaps with the Get2 binding site seems fixed, while the other interface "slides" along the second Get3 subunit during opening of the dimer. This interaction interferes with nucleotide binding and seems to induce nucleotide release. Despite these differences, both studies describe the same contribution of the Get1/Get2 cytoplasmic domains to TA protein targeting and insertion: Get2 tethers the Get3-TA complex to the membrane, while Get1 serves in positioning Get3 at the membrane for TA-protein insertion. When bound to Get3, Get1 and Get2 still allow for conformational changes in Get3. They seem to "read" the TA-protein loading state of Get3 to coordinate membrane insertion.

The current data suggest that TA-protein binding to Get3 induces ATP hydrolysis and participates in stabilization of the

transition state; however, what triggers TA protein release, when exactly ATP is hydrolysed, and whether the transmembrane part of the receptor plays an active role in insertion is still unknown. At this point, ATP-binding cassette (ABC) transporters might provide a conceptual framework also for the mechanism of TA protein insertion. ABC transporters consist of two domains, the nucleotide binding domain (NBD) and the membrane-embedded domain, which are interconnected by a so-called coupling helix (Hollenstein et al., 2007). They serve as prototype for a general "alternating access and release" paradigm of membrane pumps conceived already half a century ago (Jardetzky, 1966), which couple the translocation of diverse substrates across cellular membranes to the hydrolysis of ATP (Hollenstein et al., 2007). The following model is proposed (Figure 1): According to the Get3-receptor structures, the TA protein may be released into a shielded compartment formed at the membrane by the TABD, the Get1-CD, and the membrane-embedded part of the receptor. TA protein release disassembles the binding groove and Get3 relaxes to the closed state, as inferred from previous Get3 structures. The active site is now partially solvent accessible, so that the hydrolyzed phosphate could leave. As for the famous power stroke in muscle contraction (Sweeney and Houdusse, 2010), phosphate release via a "backdoor" mechanism might then drive the opening of the Get3 homodimer. In the open state, the active site is solvent accessible and ADP·Mg^{2+} could readily leave. Subsequent rebinding of ATP is shown to release Get3 from the membrane and allows it to enter the next targeting cycle. Interestingly, Get1-CD contains a helical turn at the tip of the coiled coil reminiscent of the coupling helices in ABC transporters. This helix interferes with the switch regions and especially with the magnesium binding site. As the CDs are rigidly linked to the TMDs, it seems plausible that the observed Get3 "gymnastics" are directly transferred to the membrane-embedded part of the Get1/2 receptor. At present, there is however no experimental evidence for such an assisted mechanism of TA protein insertion, and further work is needed to elucidate how the ATPase cycle is linked to TA protein binding and release.

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